# THE EFFECT OF HYPOPHYSECTOMY ON SODIUM METABOLISM OF THE GILL AND KIDNEY OF FUNDULUS KANSAE[1]

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If a euryhaline teleost is to maintain a reasonably constant internal environment when in fresh water or in sea water, the regulatory mechanisms operating in one environment must be capable of altered function when the animal moves into the other environment. Thus, the gill must convert from a site of ion uptake to one of ion excretion, and the kidney, which functions primarily in excreting excess water in dilute environments, must reduce its function to a minimum in the other situation. The degree and rate at which a euryhaline teleost can accomplish such alterations will determine, in part, how rapidly and how successfully transfers from one environment to the other can be made.

It now seems well established that euryhaline teleosts can reduce urine flow markedly in sea water (Holmes, 1961; Stanley and Fleming, 1964a, 1964b; Sharratt *et al.,* 1964; Fleming and Stanley, 1965), and that the reduction is due in part to a reduction in glomerular filtration rate (Holmes and McBean, 1963; Sharratt *et al.,* 1964; Stanley and Fleming, 1964a; Fleming and Stanley, 1965), and to an increase in the tubular reabsorption of water (Sharratt *et al.,* 1964; Fleming and Stanley, 1965). Further, rates of chloride and sodium flux have been shown to increase several times where a euryhaline teleost is moved from fresh water to sea water (Mullins, 1950; Motais, 1961; Gordon, 1963; Motais and Maetz, 1964, 1965).

A few reports of measurements comparing sodium fluxes across the gill with renal sodium loss, have appeared (Maetz, 1963; Maetz *et al.,* 1964; Bourquet *et al.,* 1964; Motais and Maetz, 1965), but such measurements for a single species in fresh water, during the course of adjustment to sea water, and after several days in sea water, have not, to our knowledge, been reported.

We wish here to report the results of such studies, and to describe the effects of hypophysectomy.

## Materials and Methods

The euryhaline killifish, *Fundulus kansae,* was collected from a salt spring "Boonslick" located in Howard County, Mo. The routine handling to these animals, the preparation of sea water (1000 mOsm./kg.) and the techniques used for hypophysectomy have been described elsewhere (Fleming *et al.,* 1964; Stanley and Fleming, 1964b, 1966; Fleming and Stanley, 1965) and need not be repeated here. All experiments were carried out at 19 ± 1° C., and only females which

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had been adapted to fresh water for at least two months were used. The fish selected all weighed approximately 2 grams.

Techniques for the collection and sampling of urine have also been described in detail elsewhere (Fleming and Stanley, 1965) and need only be summarized here. Urine was collected in a calibrated polyethylene cannula tied into the urogenital papilla. Urine volumes were estimated by reading directly from the calibration marks on the collection cannula. Figure 1 shows one of 10 separate compartments in an apparatus used to hold the fish relatively immobile during the experiment. A constant flow of 30 ml. per hour through each 24-ml. compartment was maintained by using a metering pump to remove water and a siphon from a constant-level reservoir to replace the water removed. Such an arrangement serves to

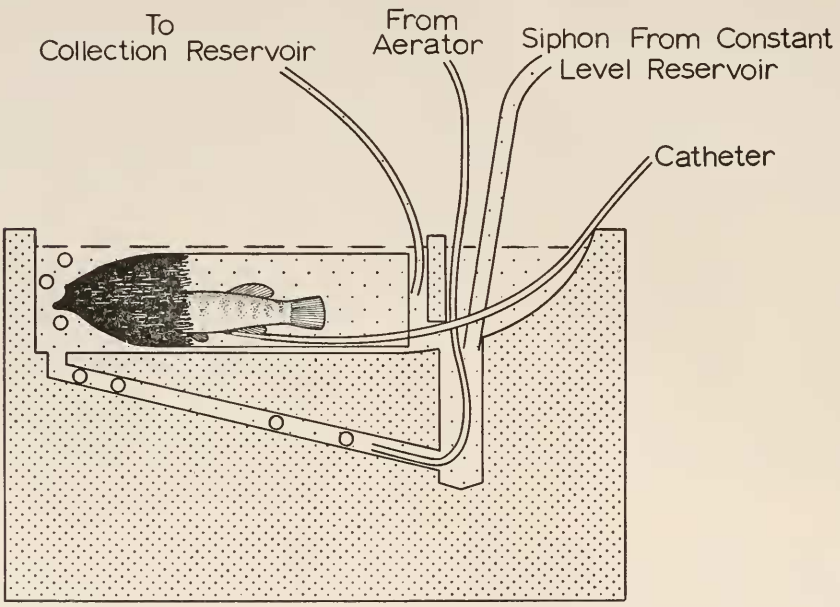


FIGURE 1. Cross-section through one of the units used to study the sodium metabolism of *F. kansae*. Urine sodium is collected in the catheter; that from other sites is carried to a collection reservoir.

separate kidney and gill excretion, and to provide a steady flow of water through the system, thereby reducing the possibility that any isotope excreted by the gill would be recycled. The water entering each compartment *via* the siphon was aerated, and each compartment was provided with a separate air line to further insure adequate aeration and to provide mixing. The water flowing through the chamber was collected in a collection reservoir.

As soon as a cannula had been secured, each fish was given an intraperitoneal injection of $Na^{22}$ carried in fish Ringer's. Each animal received 4 microcuries of isotope carried in a volume of 7.5 microliters. A micrometer-driven syringe was used to control the volume injected.

At desired intervals, samples of urine and of the fluid bathing the gills were

taken. Urine was withdrawn from the collection cannula by carefully threading a length of polyethylene tube inside the collection cannula and applying gentle suction. Samples were removed every three hours in fresh water and every six hours in sea water. The entire quantity of urine produced for each time period was blown into three milliliters of 0.02% Sterox solution. The radioactivity of this sample was determined by the use of a deep well scintillation counter. The total urine sodium was then determined on the same sample by flame photometry and the specific activity of each sample calculated.

The collection reservoir was sampled, the volume measured, and the reservoir emptied every three hours. A 3-ml. sample was counted and the total radioactivity lost *via* the gill over the three-hour period determined by multiplying by one-third the volume (in milliliters) pumped through the chamber. In every case, the counting error was kept to within 3%.

As mentioned above, renal sodium loss was measured directly with flame photometry. The extra-renal (gill) sodium loss for any time interval was determined by the equation:

$$\text{Gill loss} = \frac{\text{Urine loss} \times \text{Total gill counts}}{\text{Total urine counts}}$$

The use of this equation is based on the assumption that the ratio: sodium-22/ sodium-23, is identical for sodium lost *via* the kidney and *via* the gills.

Three separate types of experiments are reported. Experiments 1a and 1b were carried out using fresh-water-adapted animals that were cannulated, injected, and placed into fresh water. Twelve hours later, the animals were switched to sea water. Experiments 2a and 2b were carried out entirely in sea water, using animals that had been placed into sea water 8 days previously. In experiments 1a and 2a, only sham-operated animals were used; both sham-operated and hypophysectomized animals were studied in experiments 1b and 2b. In both sets of experiments, the behavior of the sham-operated animals was similar, and the data from these animals were combined. A total of 9 controls and 6 hypophysectomized animals were studied in fresh water and during the initial course of adjustment to sea water. Nine controls and seven hypophysectomized animals were examined in experiments 2a and 2b.

Experiment 3 compared the rate of sodium-22 uptake of sham-operated and hypophysectomized animals held in fresh water. A series of 8 flasks were set up, and 40 ml. of tap water containing $Na^{22}$ were added to each flask. The isotope solution was such that each initial sample provided approximately $10^4$ cpm. Two fish were weighed and placed into each flask. Enough additional solution was added so that the final volume was exactly 15 times the weight of the fish. Three-milliliter samples were withdrawn at each sampling period, counted, and returned to the flask.

## Results

### *Urine excretion*

The changes in urine flow measured when *F. kansae* was transferred from a dilute environment into sea water were largely similar to those reported in an earlier paper (Stanley and Fleming, 1966); therefore, detailed data will not be

given here. Immediately prior to transfer, the controls were excreting urine at a rate of 330 ml./kg./day, and the hypophysectomized animals at a rate of 220 ml./kg./day. Both groups reduced urine flow to approximately the same value, *i.e.*, 20 ml./kg./day, within a few hours after transfer into sea water. The same levels of urine excretion were measured for both groups after an 8-day adaptation period to the saline environment. One difference was noted from the earlier

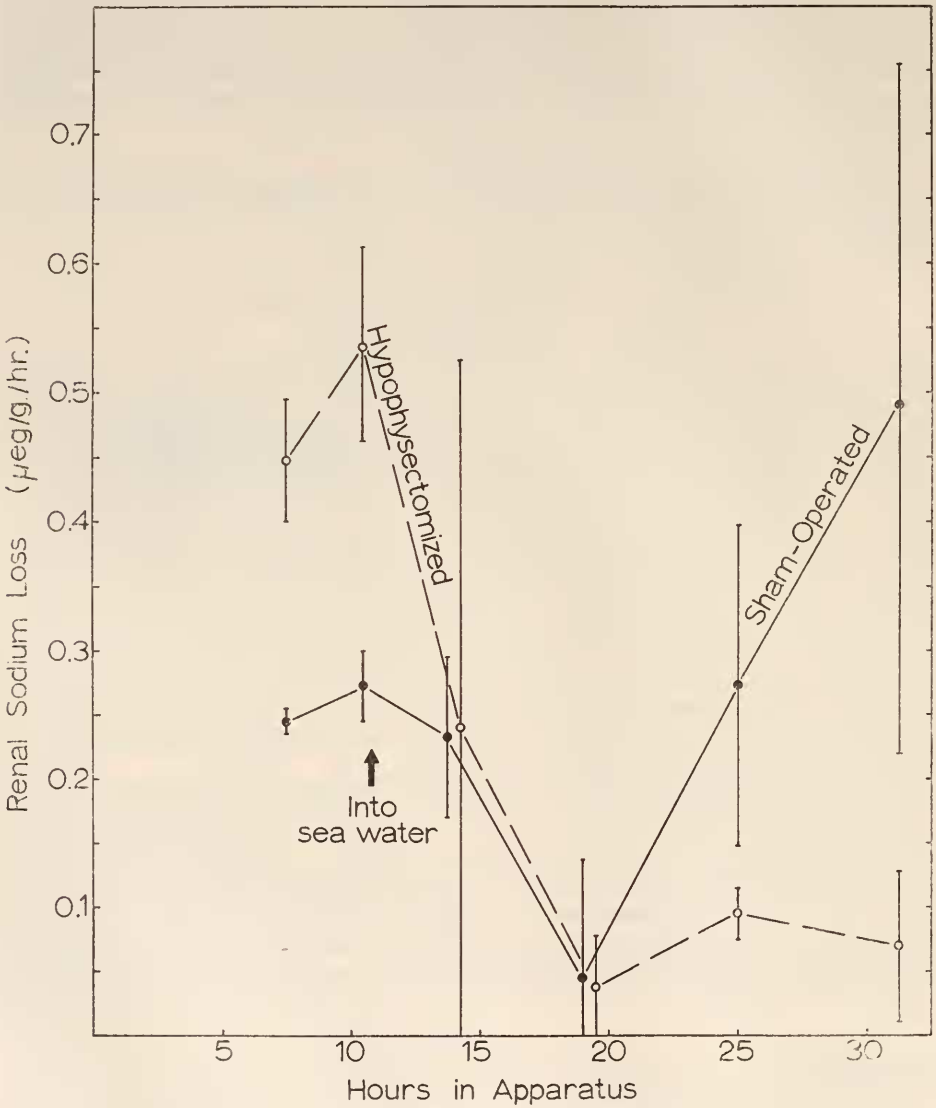


FIGURE 2. Comparison of renal sodium loss of sham-operated controls and hypophysectomized *F. kansae* held in fresh water and during the initial course of adjustment to sea water. Data show the mean ± S.E. for 9 control and 6 hypophysectomized animals.

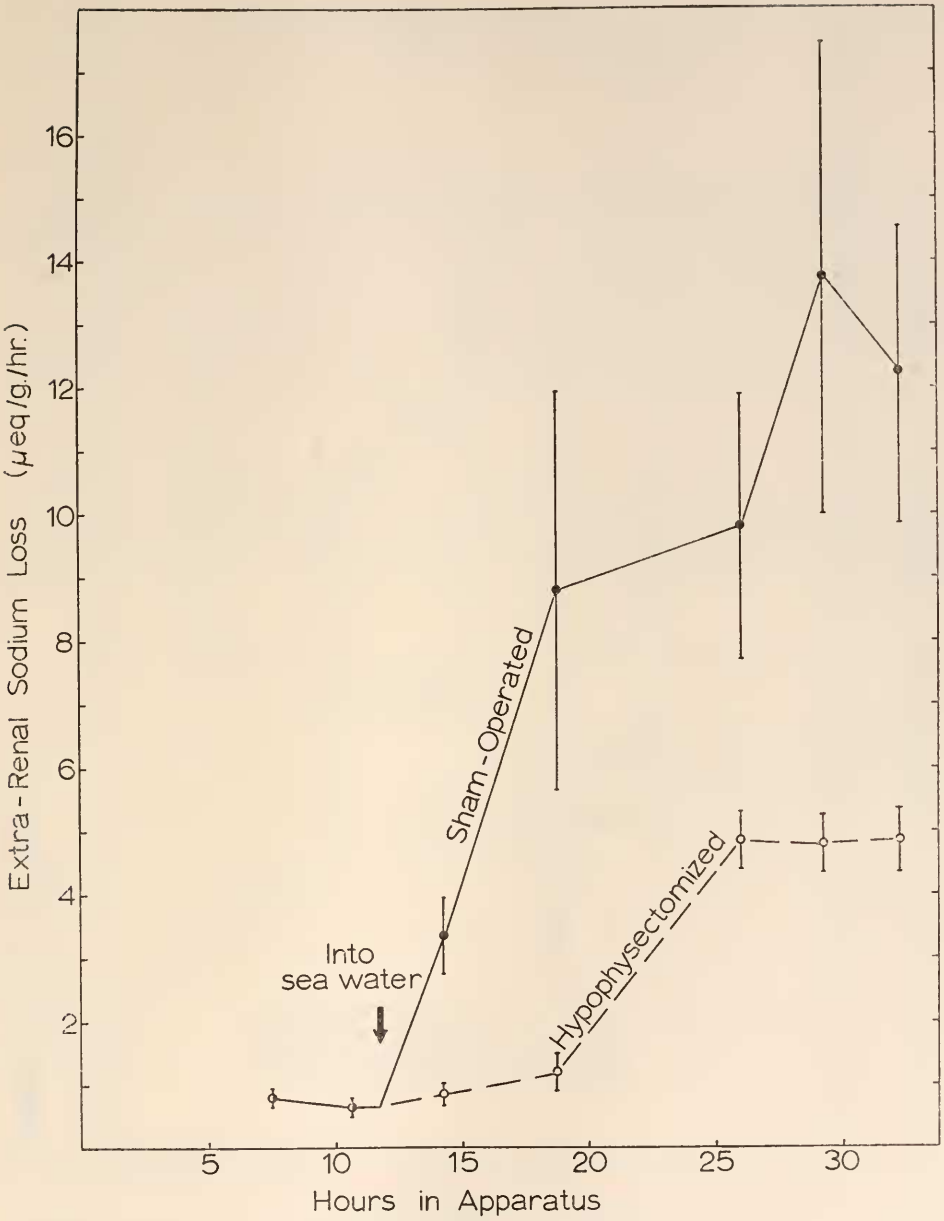


FIGURE 3. Comparison of extra-renal sodium loss of sham-operated controls and hypophysectomized *F. kansae* in fresh water and during the initial period of adjustment to sea water. Data show the mean ± S.E. for 9 control and 6 hypophysectomized animals.

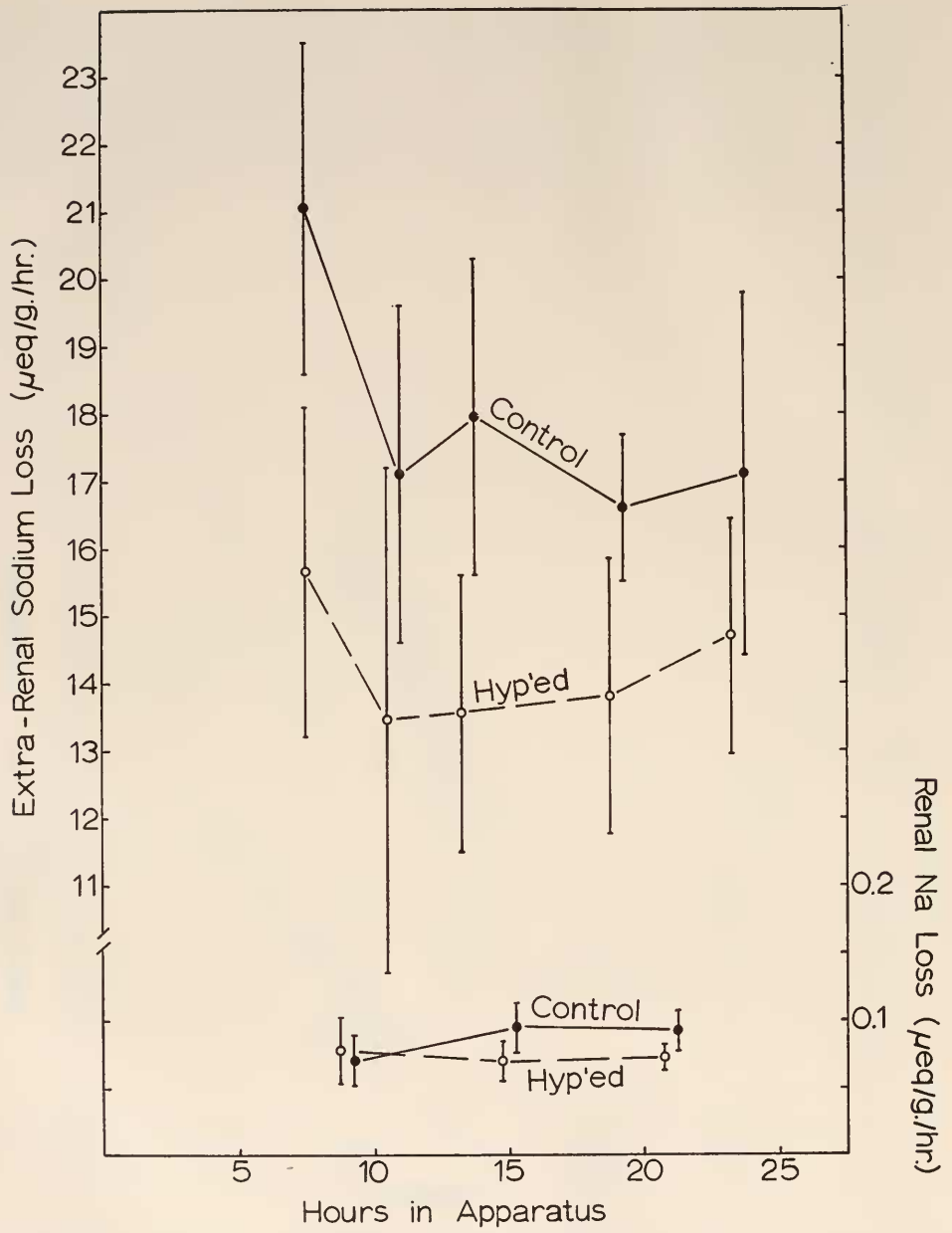


FIGURE 4. Renal and extra-renal sodium loss of sham-operated and hypophysectomized *F. kansae* after an 8-day exposure to sea water. Data show the mean ± S.E. for 9 control and 7 hypophysectomized animals.

experiments, *i.e.*, the hypophysectomized animals were able to reduce urine flow at essentially the same rate as the controls. Thus, hypophysectomy affects the rate of urine excretion of *F. kansae* in fresh water, but not in sea water.

*Renal sodium loss*

As shown in Figure 2, the renal sodium metabolism of the two groups differs markedly, both in fresh water and in sea water. Thus, the mean renal sodium loss of the control groups in fresh water was 0.27 μeq./gm./hr., for the hypophysectomized animals the mean figure was 0.53 μeq./gm./hr. Renal sodium loss fell to a low figure, 0.04 μeq./gm./hr. for both groups shortly after transfer into sea water and the hypophysectomized animals remained low there-after. The control groups showed a different response in that renal sodium loss soon increased, and by 20 hours after transfer had reached a mean value of 0.48 μeq./gm./hr.

*Extra-renal sodium loss*

Contrary to the renal picture, hypophysectomy did not affect the extra-renal sodium loss of fish held in fresh water (Fig. 3). A marked difference was clearly evident, however, when the two groups were transferred into sea water. As shown in Figure 3, both groups showed a marked stimulation of sodium outflux

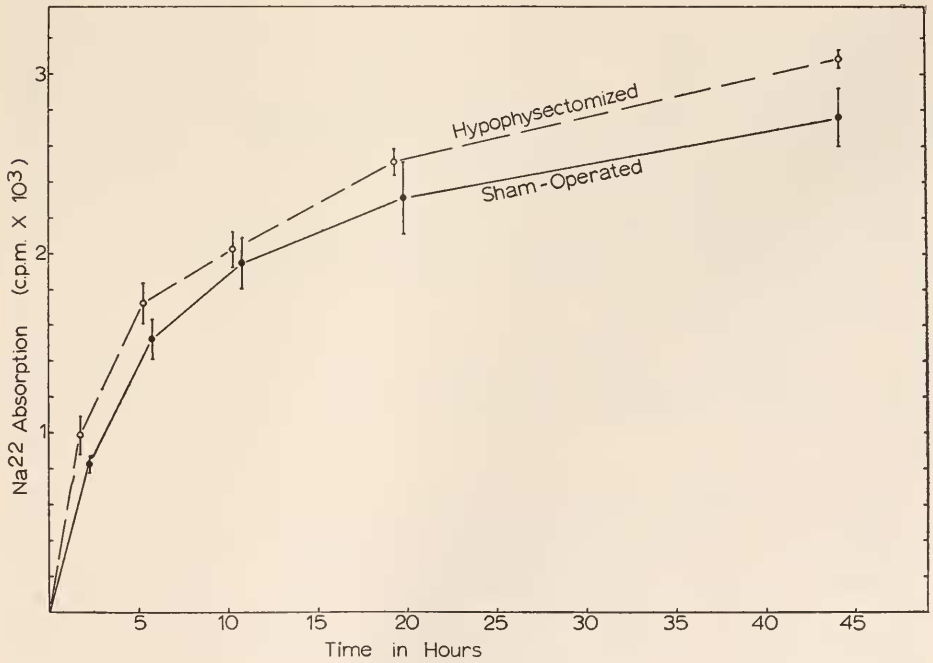


FIGURE 5. Sodium uptake of sham-operated and hypophysectomized *F. kansae* held in fresh water. Data show the counts per minute/ml. absorbed from a medium containing $10^4$ cpm/ml. and 40 meq. Na/gm. of fish. Each point shows the mean ± S.E. of four pairs of fish.

after transfer, but the response shown by the hypophysectomized animals was considerably less than that shown by their controls.

*Sodium loss after 8 days in sea water*

Figure 4 compares both renal and extra-renal sodium loss of control and hypophysectomized animals after an 8-day adjustment period to sea water. Comparisons of Figures 2, 3, and 4 show that after 8 days in sea water, both groups of fish had the same low rates of renal sodium loss. On the other hand, extra-renal sodium loss had increased for both groups, with the hypophysectomized animals still showing somewhat lower values.

*Sodium influx*

Sodium influx is slightly higher for hypophysectomized animals than for their controls (Fig. 5). The disappearance of radioactivity from the environment is rapid at first and then levels off, presumably because of recycling of isotope. Influx in the controls for the first 5½ hours was estimated by multiplying the fraction of radioactivity absorbed (1/6.5) by the sodium content of the medium (40 μeq./gm. of fish) to give a value of 1.1 μeq./gm./hr. A similar estimate in hypophysectomized fish gives a value of 1.25 μeq./gm./hr.

## Discussion

As pointed out elsewhere (Fleming and Stanley, 1965), the fact that *F. kansae* is a small fish means that a relatively large proportion of the body surface consists of water-permeable surfaces, *i.e.,* gills and oral membranes. Thus, a relatively copious urine flow, when compared with data on larger teleosts, is not surprising. A copious urine, however dilute, could provide a major site for sodium loss, and such is certainly the case for the plains killifish. Approximately 25% of the total sodium loss can be attributed to the renal route when this teleost is held in fresh water. *F. kansae* has no difficulty in remaining in sodium balance, however, and our estimates of sodium influx balance well with total sodium loss.

According to our estimates, *F. kansae* turns over approximately 1.0 μeq. $Na^+$/gm./hr. in fresh water. This figure contrasts sharply with that estimated from animals that had been exposed to sea water for 8 days. Under such conditions, renal sodium loss is negligible (0.6% of the total), and sodium influx would approximate outflux, *i.e.,* 17 μeq./gm./hr.—a 17-fold increase over the values estimated in fresh water.

It is also possible to estimate gill influx for the first few hours after transfer. During the first 12 hours in sea water, gill outflux averaged 7 μeq./gm./hr. During this same period, total body sodium rose from 62 to 132 meq./kg. body weight (Stanley and Fleming, 1965), an average of 6 μeq./gm./hr. The net increase in body sodium plus the outflux provides an estimate of sodium influx, *i.e.,* 13 μeq./gm./hr. for the first 12 hours in sea water, which is slightly more than a 13-fold increase in sodium influx over the fresh-water value.

Comparisons, then, of the estimates of sodium influx show a 13-fold increase over the fresh-water value for the first 12 hours in saline, and a 17-fold increase

for those fish held in sea water for 8 days. Sodium excretion, on the other hand, was only 7-fold higher during the first 12 hours after transfer, in contrast to the 17-fold increase estimated for the 8-day fish. These figures indicate that total body sodium must rise following a transfer to sea water, and indeed it does (Stanley and Fleming, 1965).

Both target organs respond promptly to the transfer into sea water, but the nature of the response is somewhat different. Thus, the kidney response was diphasic, sodium loss first falling from 0.28 μeq./gm./hr. to 0.04 μeq./gm./hr., and then rising sharply to 0.49 μeq./gm./hr. 20 hours after entering the saline environment. Unfortunately, the rapid loss of isotope in sea water made it impractical to continue these experiments for a longer period. It seems not unlikely that this figure would continue to increase, since this teleost can excrete a blood-hypertonic urine for a limited time (Stanley and Fleming, 1964b; Fleming and Stanley, 1965). While a figure of 0.5 μeq./gm./hr. may seem low, it is, nevertheless, sufficient to remove nearly 10% of the total body sodium over a 24-hour period. The low rate of renal sodium loss in animals immediately after transfer and in sea water for eight days can be ascribed to a low rate of urine formation.

It should be pointed out that any measurement of flux includes an error equal to exchange diffusion. In the present experiments, it is possible to place an upper limit on the magnitude of this error. Exchange diffusion should be approximately equal for all animals in sea water regardless of previous history. Exchange diffusion would then be less than the lowest outflux measurement, *viz.,* less than 5.0 μeq./gm./hr. as measured for hypophysectomized fish after initial adjustment to sea water (Fig. 3).

Previous experiments (Stanley and Fleming, 1966) have suggested a negative sodium balance for hypophysectomized *F. kansae* held in fresh water, *i.e.,* such animals had significantly less total-body sodium than did their controls. The data presented here suggest a negative sodium balance after hypophysectomy, and ocalize the metabolic fault at the kidney level. Thus, no differences in extra-renal sodium loss were observed, and the hypophysectomized fish took up sodium at a slightly higher rate than did their controls. The increase in influx, however, is not sufficient to compensate for renal loss, *i.e.,* 0.54 *vs.* 0.27 μeq./gm./hr. Although hypophysectomized killifish will live for several weeks in tap-water without food, it is necessary to provide additional sodium in their diet if they are to be held for any extended period. We have held hypophysectomized animals in fresh water for several months without difficulty, by feeding a commercial fish chow supplemented several times each week by frozen brine shrimp.

A comparison of Figures 3 and 4 suggests that hypophysectomy also affects the sodium metabolism of the gill, at least during the course of initial adjustment to sea water, *i.e.,* sodium outflux does not increase at the rapid rate shown by the control animals. After 8 days in sea water, extra-renal sodium loss is still 20% lower than in controls (Fig. 4). Hypophysectomy also affects kidney function during adjustment to sea water, *i.e.,* hypophysectomized fish do not produce hypertonic urine and do not show any increase in renal sodium loss following transfer (Fig. 2). Renal function is similar in the two groups after 8 days in sea water (Fig. 4). Thus, hypophysectomized fish appear to be less efficient in adjusting to sea water because both gill and kidney function are altered, but are capable of

sea-water-adaptation and by 8 days there are no significant differences in renal or extra-renal sodium metabolism between the two groups.

It has long been known that the European eel (*Anquilla anquilla* L.) can survive in fresh water after hypophysectomy (Fontaine *et al.,* 1949), and several studies dealing with the effect of such treatment on the sodium metabolism of this teleost have appeared recently (Chester Jones and Bellamy, 1964; Leloup-Hatey, 1964; Chester Jones and Henderson, 1965; Chester Jones *et al.,* 1965). Contrary to the data reported here for *F. kansae,* it appears that the eel remains in relatively close sodium balance after hypophysectomy, for such animals can survive in distilled water for some time—an environment that the plains killifish cannot tolerate for more than a few days at best (Pickford *et al.,* 1966). The eel also shows a marked drop in urine flow after hypophysectomy but urine sodium levels are not affected, *i.e.,* renal sodium loss is actually reduced. In contrast to *F. kansae,* the animals remain in sodium balance by reducing sodium uptake. Such data do not imply that electrolyte metabolism has not been affected, and it is clear that such is not the case, for hypophysectomized eels held in fresh water do show a drop in serum electrolytes (Leloup-Hatey, 1964; Chester Jones and Henderson, 1965; Chester Jones *et al.,* 1965).

## Summary

1. Renal and extra-renal sodium loss was measured for intact and hypophysectomized *Fundulus kansae* in fresh water and during adaptation to sea water.

2. In fresh water, urine was copious and dilute but a major route of sodium loss.

3. Following transfer to sea water, urine flow was reduced and extra-renal sodium excretion increased. Renal sodium loss decreased (because of a reduction in urine flow), then increased to above fresh water values, then, after several days in sea water, returned to a low value.

4. Hypophysectomized fish in fresh water had a reduced urine flow, an increased renal sodium loss, while extra-renal sodium outflux was unaffected.

5. Following transfer to sea water, hypophysectomized fish shut-down urine flow and although they increased extra-renal sodium excretion, they did not do so as rapidly as controls. Urine sodium loss was reduced and remained low.

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